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Phytotoxic peroxidizing actions of cyclohexanedione (RWH-series) derivatives

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Abstract: 2-(2-Chloro-5-propyloxycarbonylphenyl) aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (RWH-21), a new photo-bleaching compound, stimulates accumulation of 13²-hydroxychlorophyll *a* in cultured tobacco cells under light and dark conditions. This is based on isolation of 13²-hydroxychlorophyll *a* from pigment extracts of cultured tobacco cells by HPLC and subsequent instrumental analysis. RWH-21 causes lipid peroxidation in tobacco cells, as assessed by the formation of malondialdehyde (MDA) and ethane. Sodium azide, a singlet oxygen scavenger, partially inhibited MDA formation and the bleaching activity of RWH-21 in tobacco cells. Diphenylether-resistant tobacco cells (YZI-1S) did not show tolerance to RWH-21. Our data indicate that RWH-21 has a different mode of action from diphenylethers, and that compounds inducing the accumulation of 13²-hydroxychlorophyll *a* in plant tissues could exhibit photo-bleaching herbicidal actions.

Keywords: cyclohexane-1,3-dione derivatives; bleaching herbicide; lipid peroxidation; chlorophyll catabolism; 13²-hydroxychlorophyll *a*

Peroxidizing herbicides, such as diphenylethers (DPEs), have been the subject of considerable research because some of them are effective at very low rates (0.2–2.0 kg ha^{-1}), providing an excellent technology for weed control. One of the most notable effects of these compounds is that they stimulate the accumulation of protoporphyrin IX in plant tissues.[1] Protoporphyrin IX is a strong photosensitizer, generating singlet oxygen which is highly toxic to plant life. Many studies on the mode of action of DPEs have predicated that their primary site of action is protoporphyrinogen oxidase (Protox).[2]

We have reported that RWH-21 [2-(2-chloro-5-propyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione] exhibits DPE-like herbicidal activity in cultured tobacco cells but does not inhibit Protox *in vitro*.[3] In order to investigate the mode of action of RWH-21, we examined its effect on the induction of the accumulation of porphyrins in tobacco cells. The reversed-phase HPLC chromatograms of pigments extracted from RWH-21-treated cultured tobacco cells incubated for 20 h (A) in the light or (B) dark, or (C) without the treatment with RWH-21 in the light, are shown in Fig 1. The HPLC profiles show the elution time period from 20 to 30 min because the other parts of the HPLC profiles in three experiments were almost identical. Peaks I and III (retention time 25.7 min and 29.1 min) were assigned to chlorophyll *b* and chlorophyll *a*, respectively, by comparison of their

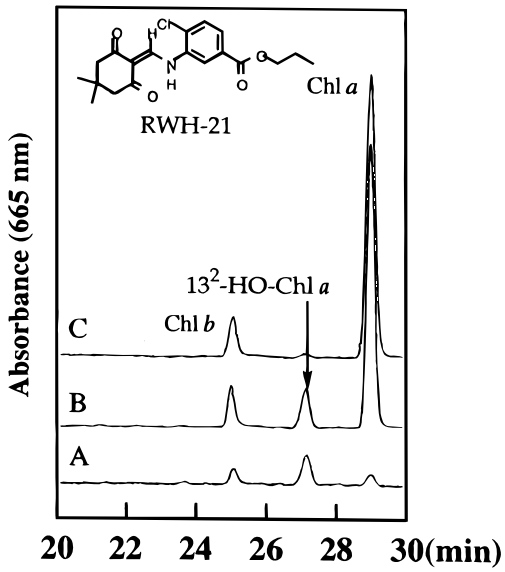

Figure 1. HPLC separation of pigment extracts from tobacco cells (*Nicotiana tabacum L* cv Samsun NN; cell line NI). Six-day-old tobacco cells were placed into a fresh culture medium containing 50 μM of RWH-21 (A) incubated in the light, (B) incubated in the darkness and (C) without treatment with RWH-21 and in the light. All experiments were done three times to give similar results. The chemical structure of RWH-21 is shown.

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retention times and fluorescence spectra with those of the authentic samples. The peak at retention time 27.2 was assigned to 13^2-hydroxychlorophyll *a* by comparison of its retention time and fluorescence spectrum with those of a synthetic standard.

Porphyrin pigments are photodynamic compounds.[4] 13^2-Hydroxychlorophyll *a* belongs to this class of compound, so it was presumed that 13^2-hydroxychlorophyll *a* induced with RWH-21 could cause photobleaching. Therefore we investigated the lipid peroxidation in RWH-21-treated tobacco cells through determination of malondialdehyde (MDA)[5] and ethane,[6] by-products of lipid peroxidation.

The time-course of MDA formation in tobacco cells is shown in Fig 2; its formation in tobacco cells within 10 h after the application of test compounds was insignificant, but the amount of MDA formed in tobacco cells increased with incubation time from 10–24 h in the light. In the dark, however, the MDA content of tobacco cells did not increase.

The time-course for ethane production in tobacco cells cultured in the presence of RWH-21 is shown in Fig 3. The production of ethane increased with the passage of time in the light. In contrast, very little ethane was formed in the dark.

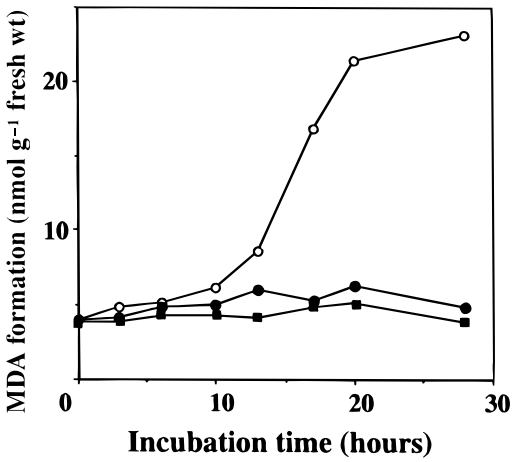

Figure 2. Time-course of MDA formation in RWH-21-treated tobacco cells. Six-day-old tobacco cells were cultured in the presence of RWH-21 at a final concentration of 50 mM (○) incubated in light, (●) incubated in darkness and (■) without treatment with RWH-21 incubated in light for the indicated periods of time. The amount of MDA was measured and quantified by a fluorescence assay of 2-thiobarbituric acid(TBA)-MDA adduct. This experiment was done three times to give similar results.

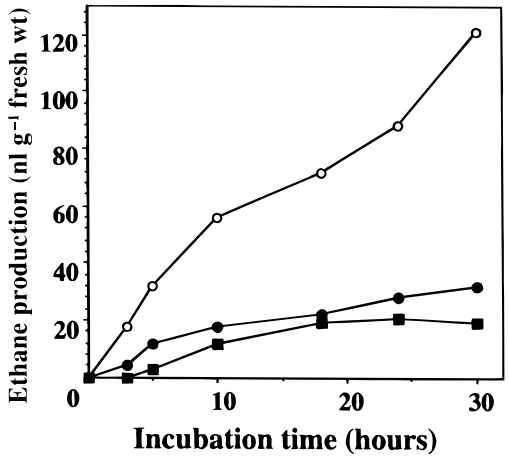

Figure 3. Time-course of ethane production in RWH-21-treated tobacco cells. The tobacco cells were incubated (○) in the presence of RWH-21 at final concentration of 50 mM in the light, (●) in darkness (■), without treatment with RWH-21 in the light. Ethane production was measured by gas chromatography. This experiment was done three times to give similar results.

In order to investigate the involvement of singlet oxygen in the herbicidal action of RWH-21, we examined the effect of free-radical scavengers on lipid peroxidation by determination of MDA formation in cultured tobacco cells. The scavengers used were: D-mannitol (for ·OH); dithiothreitol (DTT; for ·OH and O^{2-}); NaN_3 (for singlet oxygen).[7] Simultaneously, we examined the inhibitory effect of these scavengers on the bleaching activity of RWH-21 by determining the chlorophyll content in tobacco cells. As shown in Table 1, NaN_3, a singlet oxygen scavenger, partially inhibited the MDA formation and bleaching activity of RWH-21 in tobacco cells. These results suggest that the photo-bleaching activity of RWH-21 is due to the stimulation of 13^2-hydroxychlorophyll *a* accumulation, which generates singlet oxygen in the light to cause lipid peroxidation and bleaching chlorophyll pigments.

As shown in Fig 4, DPE-resistant tobacco cells (YZI-1S)[8] do not show tolerance to RWH-21. This

Table 1. Effect of free radical scavengers on MDA formation and bleaching induced by RWH-21 in cultured tobacco cells

	Light		*Dark*	
	MDA (*nmol g⁻¹ fw*)	*Chls* (*μg g⁻¹ fw*)	*MDA* (*nmol g⁻¹ fw*)	*Chls* (*μg g⁻¹ fw*)
Control	4.4(±0.4)	156(±26)	3.9(±0.3)	144(±19)
RWH-21[a]	18.5(±0.8)	2.1(±0.2)	4.5(±0.3)	139(±19)
DTT (2 mM) + RWH-21	17.6(±0.6)	0.8(±0)	9.2(±0.5)	132(±23)
D-mannitol (10 mM) + RWH-21	15.8(±0.4)	1.6(±0.1)	4.0(±0.4)	142(±21)
NaN_3(1 mM) + RWH-21	8.8(±0.4)	87(±12)	4.6(±0.3)	136(±13)

[a] Concentration of RWH-21 is 50 μM.

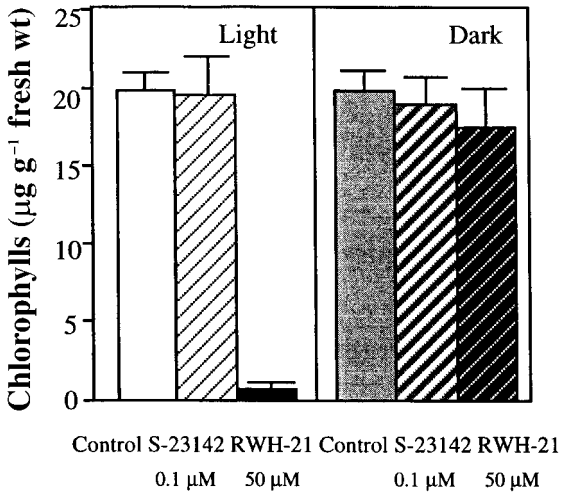

Figure 4. Bleaching activity of RWH-21 against YZI-1S cells. DPE-resistant tobacco cells (YZI-1S) were cultured in the presence of RWH-21 (50 μM) and S-23142 (1 μM), separately. Cell culture conditions are the same as shown in Fig 2.

result is in accordance with our earlier observation that RWH-21 has a mode of action different from that of DPEs.[3]

Our data indicate that compounds inducing the accumulation of 13^2-hydroxychlorophyll *a* in plant tissues could exhibit photo-bleaching herbicidal action. Thus RWH-21 could be a new lead for herbicides, and further investigation on the mode of action of RWH-21 could find a new target site of herbicides.

REFERENCES

1 Matringe M and Scalla R, Effects of acifluorfen-methyl on cucumber cotyledons: Porphyrin accumulation. *Pestic Biochem Physiol* **32**:164–172 (1988).
2 Matringe M, Camadro JM, Labbe P and Scalla R, Protoporphyrinogen oxidase as a molecular target for diphenyl ether herbicides. *Biochem J* **260**:231–235 (1989).
3 Wang JM, Asami T, Che FS, Murofushi N and Yoshida S, Photo-bleaching activity of 2-phenylaminomethylidene-cyclohexane-1,3-diones (RWH series) in tobacco (*Nicotiana tabacum*) cultured cells. *J Agric Food Chem* **45**:2728–2734 (1997).
4 Liu D, Roles of free radicals in amyotrophic lateral sclerosis. *J Mol Neurosci* **7**:159–167 (1996).
5 Li XY and Chow CK, An improved method for the measurement of malondialdehyde in biological samples. *Lipids* **29**:73–75 (1994).
6 Riely CA and Cohen G, Ethane evolution: A new index of lipid peroxidation. *Science* (*Washington*) **183**:208–210 (1974).
7 Miyao M, Involvement of active oxygen species in degradation of the D1 protein under strong illumination in isolated subcomplexes of photosystem II. *Biochemistry* **33**:9722–9730 (1994).
8 Ichinose K, Che FS, Kimura Y, Matsunobu A, Sato F and Yoshida S, Selection and characterization of protoporphyrinogen oxidase inhibiting herbicide (S-23142) resistant photomixotrophic cultured cells of *Nicotiana tabacum*. *J Plant Physiol* **146**:693–698 (1995).

Molecular shape similarity between cyclic imides and protoporphyrinogen IX

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Abstract: Comparisons using computational techniques, of shapes and bond angles of compounds which act as protoporphyrinogen IX oxidase (protox) inhibitors indicate structural similarities between the different compounds. Experiments show that cyclic imides and diphenyl ethers both inhibit protox, but have different binding sites.

Keywords: peroxidising herbicides; cyclic imides; protoporphyrinogen IX oxidase; shape similarity index

The peroxidizing compounds shown in Tables 1 and 2, which can, in a broad sense, be considered as cyclic imides – *N*-aryl-3,4,5,6-tetrahydrophthalimides (**2–11**), 4-aryl-1,2-tetramethylene-1,2,4-triazolidines (**12–16**), 5-aryl-3,4-tetramethylene-1,3,4-thiadiazolidines (**17–21**), 3-aryl-1,5-tetramethylene-hydantoins (**22**, **23**), 3-aryl-5-isopropylidene-1,3-oxazolidine-2,4-diones (**24–26**) – and peroxidizing diphenyl ethers (**27–31**) – inhibit chlorophyll biosynthesis and lead to destruction of cellular components with ethane evolution.[1,2] The target enzyme of peroxidizing compounds is protoporphyrinogen IX oxidase (protox, EC 1.3.3.4), and these compounds interact competitively with the substrate, protoporphyrinogen IX (protogen). To assess the steric similarity between peroxidizing compounds and protogen, the most stable molecular structures of some cyclic imides, diphenyl ethers and protogen were calculated and optimized by MOPAC with MNDO-PM3 parameterizations and their steric properties were compared by computational techniques.[3]

An energy-minimum conformation of protogen is shown in Fig 1 and the torsion angle of the pyrrole rings in this conformation are listed in Table 3.

For the most stable conformation of cyclic imides, the torsion angle between the imide moiety and benzene ring was approx. 240–270°, so that the conformers with the torsion angle of 255° were used in the studies of superimposition and molecular similarity as a first approximation.

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